BIOLOGICAL EFFECTS OF STATIC MAGNETIC FIELDS

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I. INTRODUCTION

Recent advances in medical instrumentation and concerns about exposure have stimulated research into the effects of magnetic fields on organisms and biological materials. There has also been a great increase in knowledge of how organisms use and are affected by the geomagnetic field. Many organisms are also known to possess inclusions of magnetic material.

There are many aspects to the effects of magnetic fields in biology. In order to make the treatment tractable, time-varying magnetic field effects have been arbitrarily separated from static magnetic field effects. Static field effects will be covered here; time-varying field phenomena including magneto-phosphenes and effects of induced currents will be covered in a companion chapter. An especially important area of growing interest concerns the biological effects and safety aspects of nuclear magnetic imaging and spectroscopy. Static magnetic fields of up to 2.0 Tesla are now used in current FDA-approved imaging systems, and several million people a year experience these fields.

Even the subject of static magnetic field effects has many aspects. These include the use of magnetic fields in spectroscopies of biological material, including nuclear magnetic resonance (NMR), mentioned above, electron paramagnetic resonance (EPR), recoilless nuclear gamma resonance (Mossbauer effect), and magnetic susceptibility and magnetization.
measurements. Magnetic fields have also been used to orient and separate cells or cell fragments in suspension. Applications of magnetism in physiology and clinical medicine include magnetic resonance imaging (MRI), magnetic targeting and modulation of drug delivery, magnetic separation of biological materials, use of magnetism in surgical procedures, and noninvasive measurement of blood flow. An important topic area spanning AC and DC magnetic field regimes is the measurement of magnetic fields generated by the human body, and the use of those measurements in medicine and physiology. A large topic area involves mutagenic, mitogenic, metabolic, morphological, and developmental effects of exposure of organisms or biological materials to intense DC magnetic fields or to null field conditions. Another important topic area includes behavioral effects of magnetic fields, including effects on orientation, migration, and hunting and the involvement of the geomagnetic field in the activities of organisms. Biomineralization of magnetic materials is especially significant for this latter topic, but could also play a role in other interactions of organisms with electromagnetic fields.

II. PHYSIOLOGICAL EFFECTS AND MEDICAL APPLICATIONS OF MAGNETISM

Magnetic properties and spectroscopy of biological materials have been extensively studied. Especially significant advances in NMR have been made. These include increased resolution and sensitivity, pulsed programming, Fourier decomposition of complex spectra allowing study of whole organisms or perfused organs, and observation of the time development of chemical intermediates, e.g., metabolites containing phosphorus, in metabolic pathways. Multidimensional NMR has been applied to the determination of protein structure in solution.

Superconducting magnets with bores large enough to accommodate human bodies have allowed development of NMR imaging systems with resolution comparable and even superior to X-ray and positron computerized tomography and ultrasound techniques. The basis of the method is that when a magnetic field gradient is superposed on a static, homogeneous magnetic field, nuclei such as protons will resonate at different frequencies at each point along the gradient. The amplitude of the signals at each frequency is proportional to the number of protons at that point. By switching the gradient to different directions and recording the spectra, a two- or three-dimensional proton density map can be reconstructed by computer. Measurement of relaxation times also allows discrimination of chemical differences, e.g., different types of tissue. Fields of the order of 5 × 10⁻² to 2.0 T with gradients of the order of 10⁻² T/m are used for periods of the order of 1/2 hr. Switching of the gradients can involve rates of magnetic field change as high as 2 T/sec.

Major safety issues associated with NMR imaging have been identified by Kanal and colleagues. Biological effects associated with static magnetic fields is one of eight safety concerns relevant to NMR imaging: others are ferromagnetic attractive "projectile" effects, biological effects of the time-varying magnetic fields (mainly induced currents), biological effects of the RF field (mainly RF heating), auditory noise caused by the pulsed magnetic fields, concerns about the cryogens used in the superconducting magnets, claustrophobia and anxiety, and complications due to the NMR contrast agents. The specific problems associated with static magnetic fields and ferromagnetic objects have been reviewed in detail. An associated safety issue relates to potential health risks to NMR imaging workers who are exposed to static and time-varying magnetic fields used in NMR. Epidemiological data was recently published in which women workers in more than 90% of clinical NMR facilities in the U.S. were evaluated for menstrual-reproductive experiences and work activities. No statistically significant differences were observed between NMR workers and the control cohort group in spontaneous abortions, conception taking more than 12 months, delivery
before 39 weeks, birth weight below 5.5 lb, or male gender of the offspring. These results suggest that a typical NMR worker environment is not a risk factor for these common adverse reproductive outcomes. A smaller but related study of the health of 11 human volunteers who experienced varying degrees of exposure to a 4.0 Tesla whole-body NMR imaging device over a 12 month period also reported no major adverse health problems associated with exposures. The only positive findings related to mild sensations of vertigo, nausea, metallic taste, and magnetophosphene production all associated with motion within the 4.0 Tesla static magnetic field.

The above findings of mild sensory effects in humans exposed to 4.0 Tesla fields may have relevance to behavioral alterations reported in rats exposed to 4.0 Tesla static fields. A simple T-maze was used to evaluate behavioral effects of rats in 1.5 and 4.0 Tesla fields. Rats were observed to enter the bore of the magnet at 0 and 1.5 Tesla freely. At 4.0 Tesla 97% of the rats would not enter the magnet and most of the decisions to turn around were made at the edge of the magnet in a region of a strong gradient field (13 T/m).

Several studies have investigated central nervous system activity in human subjects before and after exposure to NMR imaging fields up to 2.0 Tesla. Several groups have studied auditory evoked potentials in the human brainstem, in which subjects were assessed before and after routine NMR imaging at 1.5 or 2.0 Tesla, or during stepwise increments in field strength to 1.5 or 2.0 Tesla. No long-lasting, significant differences in interpeak latencies were noticed before or after NMR imaging, or during the stepwise field intensity tests. A third report describes measurements of somato-sensory evoked potentials excited from median nerve stimulation in normal human subjects before, during, and after short-term exposure to a 1.5 Tesla static magnetic field. No changes in interpeak latencies were observed. The above studies suggest that nerve conduction and synaptic transmission are within normal limits in human subjects exposed to such fields. These findings are consistent with studies discussed below (Section IV) in which static magnetic fields of 4.7 Tesla do not alter visually evoked potentials in the cat brain.

Human cognition has been investigated in volunteer, neurologically-normal, subjects who were randomly assigned to a typical NMR treatment (0.15 Tesla), sham treatment, or control treatment groups. Subjects were tested in a double-blind, prospective format at pretreatment, post-treatment, and follow-up time periods. Six different tests were administered that assessed visual retention, mental rotation (3D visualization), memory scanning, and anxiety. No significant differences were reported between treatment groups. These studies are consistent with negative findings from studies employing rats in which spatial memory, open field behavior, and passive avoidance was investigated in animals exposed to a 0.15 Tesla NMR imaging procedure. Human studies have also been conducted to assess whether hormone secretion is altered due to NMR fields. Four healthy adult male volunteers (22-35 yrs) participated in the study which involved obtaining nine hourly blood samples between 2000 and 0400hrs on two different nights, one week apart. Between 2400 and 0200 hrs the subjects were exposed to a 0.15 Tesla NMR procedure or sham treatment. Hormone levels of melatonin peak at night and lighting was maintained well below threshold levels that trigger melatonin secretion. In addition, prolactin and growth hormone were also monitored and no statistically significant alteration in these three hormone levels was reported.

A number of animal studies have been conducted using NMR imaging fields. Rats were exposed to a 0.15 Tesla NMR procedure for 23 minutes for five successive days or for twenty-one successive days and 13 to 23 months. Thereafter animals were examined for abnormalities in body weight, spleen, heart, thymus, adrenal weights, white blood cell counts, hemoglobin, adrenocorticotrophin, and cortisol levels. No evidence for changes in long-term survivability or stress was reported. A series of studies in mice and snails has shown that 0.15 Tesla NMR fields attenuate morphine-induced, and fentanyl-induced analgesia in mice. Of interest in these studies is that the time-varying magnetic field component was...
nearly as effective in eliciting the response by itself as the complete NMR field environment.\textsuperscript{42,45} The static magnetic field, therefore, is not strongly implicated as a causative factor in these studies. The authors note that these responses are small and appear to be reversible, and may operate through alterations in second messengers such as calcium and in protein kinase C activity.

Several animal studies exist which suggest a potential effect of NMR imaging fields on embryo development and cell metabolism. Concerns about NMR imaging fields influencing pregnancy outcomes was a major factor in initiating the Kanal epidemiology study, discussed above, which dealt with technical staff that experienced fringe fields during NMR imaging procedures. In contrast, patients experience the full complement of all three field components in the magnet bore. Perhaps the greatest difference in patient exposures is the increased likelihood for localized heating, a known teratogen, from the focused RF fields and time-varying magnetic fields in the magnet bore.\textsuperscript{46,47} In this regard, the combination of localized heating plus X-ray diagnostic procedures which may precede NMR imaging scans is a potential factor for consideration. When pregnant mice were exposed for 16 hours beginning on gestation day 8.75 to 0.35 Tesla NMR imaging fields, a significant reduction in crown-to-rump length was reported, with no change in resorptions, stillbirths, fetal weight, or homeotic shifts.\textsuperscript{48} In studies reported by Tyndall, the teratogenic potential of NMR imaging fields on development of the eye in mice was investigated.\textsuperscript{49} Mice were exposed to NMR imaging fields at the isocenter of the magnet (1.5 Tesla) or the entrance (0.4 Tesla) on day 7 of gestation, the most susceptible stage of eye development. On day 14, concepti were removed and eye formation was evaluated by veterinary pathological examination and by computerized morphometric analysis. Both exposure locations to the NMR fields resulted in a similar statistically significant increase in abnormal eye formations compared to controls. It is of interest that both exposure locations lead to the same increase in affected fetuses.

Exposure to static magnetic, RF, and time-varying magnetic fields are significantly different at the isocenter compared to the entrance of a NMR system. In particular, the static magnetic field at the isocenter is spatially uniform whereas at the entrance it has the characteristics of a gradient field. In other studies, using the chicken egg as a model system, NMR imaging fields of 1.0 or 4.0 Tesla did not result in alterations of embryo mortality, hatching rate or vitality of the chicken.\textsuperscript{50} NMR imaging fields at 0.15 Tesla are reported to increase the synthesis of collagen in the dentin and bone of mice, but not synthesis in alveolar and tibial bone tissue.\textsuperscript{51}

The blood-brain barrier (BBB) has been investigated in animals exposed to NMR imaging fields. At least seven laboratories have reported on this question, and, as such, it is one of the most widely investigated topics across laboratories. In addition, there has been an attempt to define which of the three field components is responsible for an effect on the BBB. In general, rats have been employed and the animals are chemorestrained so that an agent, such as a radiotracer-labeled compound, is injected into the circulation and the brain assessed for uptake of this compound following NMR imaging procedures. Technical differences such as the use or non-use of anesthesia as a chemo-restraint, the molecular size of the BBB marker compound, whether an acoustic control was employed or whether the animals were conditioned for handling,\textsuperscript{52} and whether the rat circulatory system was perfused after the experiment to flush out excess tracer, do not appear to correlate with an effect on BBB permeability. The only ostensible correlate appears to be that NMR imaging systems that operate at $< 0.15$ Tesla show a small, reproducible alteration in the blood-brain barrier.\textsuperscript{53–56} In contrast, negative findings have been reported for studies employing higher strength static magnetic fields up to 4.7 Tesla.\textsuperscript{56–59} The biological basis for such alterations in the BBB may relate to functional changes in pinocytic activity or capillary endothelium function, or to alterations in brain circulation. Since these alterations appear to be small and reversible, the clinical significance of these changes are as yet unclear.
Understanding the physical basis for these results is a challenging task since three different electromagnetic fields are present in the NMR imaging environment. In addition, the time-varying magnetic field is pulsed and may possess extremely-low-frequency (ELF) components raising the possibility that ELF magnetic fields associated with the time-varying field may be a factor in observed bioeffects.\textsuperscript{12,25,42,60} Two of the above BBB investigations have attempted to experimentally separate the field components to directly test whether the static magnetic field, time-varying magnetic field, or RF field is the operative component. Oldendorf and colleagues reported that NMR imaging procedures led to a slight, but statistically significant, increase in 3H-mannitol uptake in the brain at 0.3 and 0.5 Tesla, but not at 1.5 Tesla.\textsuperscript{56} Moreover, when field components were studied separately they report that no changes in BBB were observed for the 0.3 Tesla static field alone or the RF field alone. However, the time-varying magnetic field alone led to changes that could account for changes due to the complete field. Persson and colleagues report that the BBB is increased in rats exposed to 0.08 Tesla NMR imaging fields, but not in those exposed to 2.35 Tesla.\textsuperscript{53} To address the question of which field component was operative at 0.08 Tesla they performed separate exposures to static and time-varying magnetic fields and the RF field. Although the number of animals was small (5–6 per group) they reported a statistically significant increase in BBB permeability, as assessed by Evan’s Blue dye extravasation, for rats treated with all three components separately, however, the RF component was most prominent and similar to the complete field treatment. Studies at 915 MHz RF (CW and modulated at 8–215 Hz) using a larger cohort of rats (20–35 per group) led to statistically significant increases in the number of animals showing endogenous albumin leakage into the brain assessed by fluorescent antibody techniques (15%, controls vs. 67% exposed, \(p = 0.0001\)). The reversibility or clinical significance of these changes is at present unknown.

A small number of studies of the effects of NMR imaging fields on cellular model systems have been reported. The major advantage to using well-defined cellular systems in bioeffects studies is that biologically-based interaction mechanisms can be addressed directly using simple systems. Exposure of cells to NMR imaging fields requires specific consideration of temperature control and isolation from vibrations during exposures.\textsuperscript{2}

Two groups reported effects involving calcium metabolism in lymphocytic cells. These studies are of interest since calcium is a second messenger and plays a major role in cell growth and differentiation.\textsuperscript{25,61,62} Alteration in calcium signaling across the cell membrane is an attractive biological framework for understanding magnetic field effects on fundamental events in cells involved in the signal transduction cascade such as RNA, DNA, and protein synthesis and cell proliferation. The T-lymphocyte is one of the best characterized model systems for investigating calcium signaling.\textsuperscript{25}

When human peripheral blood lymphocytes or rat thymic lymphocytes were placed at the end positions of a 2.35 Tesla NMR imaging magnet, a significant increase in calcium-45 uptake was reported for cells undergoing signal transduction during mitogen activation.\textsuperscript{25}

For both human and rat lymphocytes the mitogen employed was Con-A used at suboptimal concentrations so that the mitogen itself did not significantly elevate calcium uptake. However, for both cells the NMR field acted synergistically to enhance calcium uptake in the presence of suboptimal levels of mitogen by approximately 30%. Thus, the NMR fields acted as a co-mitogen to amplify calcium signaling. This concept of field enhancement of mitogen activation represents a synergistic interaction that has biological significance. The effective threshold for mitogen activation was shifted to lower mitogen concentrations in the presence of the NMR fields and this suggests that the NMR fields enhanced the cells responsiveness to mitogen. Further studies with this model cell system were reported in which two field components, the 2.35 Tesla static magnetic field and the RF field, were evaluated separately: neither influenced calcium influx during mitogen activation.\textsuperscript{53} This suggests that neither the RF nor the static magnetic field was involved in the field interaction. Additional exposures...
to static magnetic fields were reported at 7.5 Tesla with no effect on mitogen-activated calcium influx, which is consistent with findings at 2.35 Tesla, but exposures at 9.0 Tesla resulted in a statistically significant decrease in mitogen-activated calcium uptake. The latter effect is in the opposite direction to that reported for 2.35 Tesla NMR imaging fields discussed above. These decreases in calcium influx in a 9.0 Tesla magnetic field may represent a static field interaction on cell membrane components that have a threshold between 7.5 and 9.0 Tesla. Calcium influx may lead to changes in free intracellular calcium, and intracellular calcium was recently reported to be elevated by approximately 30% in HL-60 lymphocytic tumor cells exposed to 0.15 Tesla NMR imaging fields. It is of interest that the field component associated with this field effect was reported to be the time-varying magnetic field. Thus the static magnetic field was not implicated. The increase in intracellular calcium observed in the complete 0.15 Tesla NMR field is consistent with an increase in calcium influx observed for lymphocytes exposed to the 2.35 Tesla NMR imaging field discussed above.

The effects of a static gradient magnetic field on cell cycle and growth rate were reported for mammalian FM3A cells. Exposure to 0.6 Tesla/m resulted in a 5% reduction in growth rate, a decrease in cells in G1 phase up to eight hours post-exposure, and a drop in cell viability.

A number of studies have reported on the effects of static and ELF magnetic fields on the pineal gland and melatonin levels in rats and other organisms. Reuss and Olcese reported that light stimulation was necessary for the sensitivity of the pineal gland and melatonin content to magnetic fields. Lechle et al. has reported that the magnetic sensitivity of the pineal gland results from induced currents. However, no connection between these effects and behavioral responses to magnetic fields have been established.

The development of superconducting quantum interference devices (SQUIDS) with very high sensitivity has spurred the study of the very weak magnetic fields generated by electrical processes in the human body. Applications include magnetocardiography, magnetoecephalography, measurement of pulmonary activity, and detection of body iron stores due to asbestos inhalation or diseases such as Thalassemia, which result in hemochromatosis. Magnetic devices have been used in several surgical procedures including repair of giant retinal tears, bougienage of esophageal atresia in infants, and in the treatment of aneurysms. Magnetic agitation has been used to modulate the release of macromolecules such as insulin from polymers with magnetic inclusions which are implanted in the body. The voltage induced when an electrolyte flows perpendicular to a magnetic field, known as the magnetohydrodynamic effect, has been used noninvasively to measure blood flow. The voltage induced across a blood vessel is proportional to the vessel diameter, the magnetic flux density and the flow velocity. A velocity of 0.6 m/sec in the human aorta and 1 Tesla result in a 15 mV potential difference. These potentials also show up in electrocardiograms of animals in magnetic fields (see Section IV).

Magnetic microcarriers have been used to target drugs to specific locations in the body and in cell separation and immunological assays. These applications are based on the translation of paramagnetic and ferromagnetic particles in the direction of increasing magnetic field, relative translation of the diamagnetic fluid background in the opposite direction. The particle velocity due to magnetic forces depends on the nature of the material, the magnitude of the field gradient, and the size of the particle. The size of magnetic microcarriers is limited by the need for a large surface-to-volume ratio, and by the tendency of large particles to coagulate due to interparticle forces.

To employ magnetic drug microcarriers, large magnetic field gradients must be generated outside the body by suitably shaped, magnetized pole pieces. In the in vitro procedures, the microcarriers, to which specific antibodies or antigens are chemically attached, can be separated by the use of high gradient magnetic separation (HGMS) filters consisting of fine stainless steel wires in a magnetic field strong enough to magnetize the wires. Gradients as
high as $10^3$ to $10^6$ T/m can be generated within a few diameters of the wires. As the fluid flows through the filter the magnetic particles and anything attached to them are trapped on the wires. This method has been applied to the separation of cells, proteins and nucleic acids\textsuperscript{87-91} and to removal of microorganisms from water.\textsuperscript{92,93}

Magnetic fields have been used to release drugs from liposome vesicles and this suggests a novel means for noninvasively triggering drug delivery.\textsuperscript{94} Liposomes are used to carry pharmaceutical agents or chemicals in the lipid bilayer or in the interior aqueous phase of the vesicle,\textsuperscript{95,96} encapsulation of highly toxic drugs is advantageous since sequestration prevents damage to normal tissue. The challenge is to direct the liposome vesicle to the appropriate target tissue and to trigger release of the agent into the target cell. Achieving the desired biodistribution has been approached in different ways; targeting by antibodies to cell surface markers,\textsuperscript{97} specialized lipid compositions to promote prolonged circulation in the bloodstream,\textsuperscript{98} and modification of liposome size to preferentially enhance target tissue specificity.\textsuperscript{99} Recently, static magnetic fields and time-varying fields have been shown to trigger the release of drugs from liposome vesicles.\textsuperscript{92,99-103} This technique permits the controlled delivery of drugs using a noninvasive physical agent such as a magnetic field; liposome depots placed subcutaneously in different areas of the body can be treated with magnetic fields to release drugs in a controlled manner into the bloodstream.\textsuperscript{103} Controlled, on-demand, temporal delivery of drugs is an evolving area of medicine that seeks to optimize the timing of drug delivery for maximal drug effectiveness.\textsuperscript{104} Mechanisms of magnetic field interaction with the synthetic phospholipid bilayer of liposome vesicles is discussed in Section III.

A novel medical application of static magnetic fields is the use permanent magnet devices for retaining prosthetic appliances. In 1972 the rare earth cobalt magnet was developed; this led to its application in dentistry.\textsuperscript{105} Sm-Co magnets have excellent magnetic properties but are not easily castable, and three new alloys were developed composed of palladium and cobalt (Pd-Co).\textsuperscript{106} Biocompatibility tests showed least cytotoxicity with a nickel composition (Pd-Co-Ni).\textsuperscript{107} Optimization schemes and design considerations have been developed,\textsuperscript{108} as well as construction techniques\textsuperscript{109} and specific dental prostheses.\textsuperscript{110,111}

### III. CELLS, BIOMOLECULES AND CHEMICAL REACTIONS IN MAGNETIC FIELDS

Alignment of molecular aggregates with sufficient diamagnetic anisotropy will occur in intense magnetic fields (see Appendix). Experimental results have been reviewed by Maret and Dransfeld,\textsuperscript{112,113} and by Gretz et al.\textsuperscript{114} Muscle fibers,\textsuperscript{115} chloroplasts,\textsuperscript{116} retinal elements,\textsuperscript{117,118} sickle erythrocytes,\textsuperscript{119,120} bacteriophage fibers,\textsuperscript{121} membranes,\textsuperscript{122,123} and macromolecules including nucleic acids\textsuperscript{112} have diamagnetic anisotropy and have been aligned in intense, homogeneous magnetic fields. Highly oriented structures can result from polymerization in an intense field. Torbet et al.\textsuperscript{124} and Freyssinet et al.\textsuperscript{125} produced oriented fibrin gels from fibrin monomers in a field of 11 T. The fibrin monomers were produced by enzymatic cleavage of fibrinogen. Murthy and Yannas\textsuperscript{126} prepared oriented collagen films by heat precipitation in a magnetic field. Collagen in solution dissociates into monomers at low temperatures and precipitates at higher temperature ($\sim 37^\circ$C). The monomers are apparently not aligned. Alignment of dimers, trimers, etc. occurs as they form monomers with increasing temperature in the field. Conversely, magnetic fields of 0.5–1.8 T were reported to disrupt cellulose polymerization in oat leaf fibrils and in the bacterium Acetobacter xylinum.\textsuperscript{114}

In addition to polymer alignment, intense magnetic fields may interact with biomaterials in other ways. Sperber et al.\textsuperscript{127} observed oriented growth of pollen tubes in intense fields and suggested a redistribution of membrane proteins that regulate intracellular concentrations of calcium. Audus\textsuperscript{128,129} previously observed oriented growth, or magnetotropism, in oat shoots and cress roots in inhomogeneous magnetic fields, with growth occurring in the
direction of decreasing field intensity, Labes\textsuperscript{130} and Aceto et al.\textsuperscript{131} proposed interaction of magnetic fields with cell membranes as a plausible mechanism for physiological effects. They noted that membranes have liquid crystal-like properties and are close to phase transitions at physiological temperatures. Magnetic orientation of diamagnetically anisotropic domains in artificial phospholipid bilayers has been reported.\textsuperscript{132} Magnetic fields also affect the fluid-gel transition in agarose gels,\textsuperscript{133} possibly by alignment of the monomers in the fluid phase.

There are reports of alteration of enzyme activity \textit{in vitro} by magnetic fields.\textsuperscript{134,135} For example, Haberditzl\textsuperscript{134} reported that fields up to 7.8 T diminished the activity of glutamic dehydrogenase, while a 6 T field enhanced the activity of catalase. Nonuniform fields produced larger effects than uniform fields. Weissbuth and co-workers\textsuperscript{136} reported no effects of intense magnetic fields up to 22 T on the activities of several enzymes.

Because of the important role that the cell membrane plays in mediating interactions with static magnetic fields, a number of studies have been undertaken to investigate how simple synthetic lipid bilayers interact with static magnetic fields. In these studies, the liposome vesicle is used as a model system for the natural cell membrane since the phospholipid bilayer represents a simple bilayer membrane structure that can be made more complex by incorporating, for example, protein receptor ensemblers and ion channels structures. Unilamellar liposome systems are the simplest bilayer systems for use in laboratory investigations. An important feature of such single bilayer systems is that they display well-defined structural changes in organization during phase transitions.\textsuperscript{101} This appears to be important to static magnetic field interactions since profound structural changes in phospholipid organization, e.g. changes in packing density of hydrophobic hydrocarbon tails in the bilayer and of polar headgroups at the membrane surface, as well as lateral compression of the bilayer, will influence the magnetic susceptibility tensor which determines the magnitude of the static field interaction.\textsuperscript{2,101} Such changes in organization of the bilayer are relevant to functional aspects of natural cell membranes.\textsuperscript{137}

Previous studies of dipalmitoyl lecithin vesicles in 3.8 Tesla static magnetic fields have reported changes in magnetically induced birefringence at temperatures above the phase transition temperature, $T_c$.\textsuperscript{138} This was interpreted as reflecting lipid orientation in the bilayers. Light scattering studies on liposomes of various compositions have also reported that changes in turbidity occur during exposure to static fields greater that 0.2 Tesla at temperatures near or at the phase transition. These studies were also interpreted as reflecting membrane phospholipid orientation.\textsuperscript{139} In addition, other studies at 9.3 Tesla indicate that phospholipid orientation at temperatures below the phase transition in the gel phase does not occur.\textsuperscript{140} The above studies raise an interesting question: are field effects on structural changes such as phospholipid orientation, which are dependent on $T_c$, able to change membrane transport properties of the bilayer? This relates directly to a functional property of natural membranes.

This question was addressed in experiments in which unilamellar liposome vesicles were loaded with a radiolabeled aqueous phase marker, tritiated arabinofuranoside (3H-ARA-C), in the interior of the liposome, and solute release was assessed during exposure to a range of static magnetic fields and temperatures.\textsuperscript{94} These liposomes exhibited enhanced solute release during brief (15 minute) exposures to 7.5 Tesla magnetic fields at temperatures near but lower than the characteristic phase transition temperature of $\sim$41C (Figure 1). Kinetic studies at 7.5 Tesla revealed that the magnetic field approximately doubled the rate of solute leakage across the bilayer essentially instantaneously and that a slow rise in this permeability increase occurred over a 40-minute period (Figure 2).

These data indicated that magnetic field-induced, membrane permeability changes are possible in simple phospholipid bilayers, and that these field interactions display a dependence on the structural organization of the membrane.
An approach for analyzing magnetic field interactions with cell membranes, based on molecular dynamics, takes into account changes in the local curvature of the bilayer. The groundwork for this approach was originally developed by Helfrich\textsuperscript{141} and was later applied to liposomes.\textsuperscript{101} This approach rests on the observation that membrane structures undergo oscillations or vibrations (dynamic fluctuations) at the molecular level that are dependent on density packing of phospholipid elements in the aggregate structure.\textsuperscript{142} Molecular dynamic fluctuations spontaneously produce local regions of curvature in the bilayer.\textsuperscript{113} Such structures have been identified in liposomes and these local areas of molecular curvature in the bilayer are known as ripple structures which are formed spontaneously at pretransition temperatures.\textsuperscript{144}

The idea of a singularity at which the cell membrane becomes unstable and is extremely sensitive to physical deformation by the application of an external magnetic field provides a theoretical basis for low field intensity interactions which are not addressed by classical thermal interaction analysis. Natural cells change shape during growth and differentiation. These changes in morphology may potentially predispose the cell to magnetic field deformation; such an interaction would be expected to exhibit cell-cycle dependence. In addition,
natural cell membranes exhibit phase separations and lipid clustering that give rise to the presence of lipid domains. These changes in membrane organization or phase transitions are associated with profound changes in enzyme activity and ion transport, usually at or near physiological temperatures. Local changes in lipid-protein packing, bilayer compressibility, and membrane fluidity would all play a role in modulating the local, spontaneous curvature of the cell membrane and, thus, the environment of the enzyme or ion channel.

At the surface of the cell a number of important processes occur that are critical to cellular function such as receptor binding events and the transport of second messenger ions, such as calcium, through ion channel structures in the bilayer. The effects of static magnetic fields have been reported on these processes in a number of model systems. Static magnetic fields of 2.0 Tesla, comparable to that used in NMR imaging systems, were used to investigate the binding of ligand to the acetylchoine receptor (AChR) in vitro. Binding of the competitive antagonist, alpha-Bungarotoxin, to AChR in the magnetic field was significantly reduced at 45 minutes compared to control treatment. This effect was reversible and did not decrease the total number of available binding sites since maximal binding (as in controls) was achieved after 2.75 hours in the field. Pretreatment of AChR in the magnetic field for 2.5 hours had no effect on ligand binding. Thus, this interaction required the presence of both ligand and receptor, and apparently influenced the initial rate of receptor-ligand complex formation. Aoki and colleagues studied the effects of a 0.4 Tesla static magnetic field on adriamycin (ADR) movement across the cell membranes of cultured TALL-1 cells. A fifteen minute field exposure was reported to result in an increase of less than 5% of ADR accumulation observed in the sham-treated cells. Experiments were carried out within a temperature range of 41–46°C corresponding to the phase transition temperature, suggesting that lipid ordering and bilayer structural organization, as discussed above, play a role in this interaction. Other studies implicate calcium movement across the cell membrane as a factor in interactions with static magnetic fields. Human polymorphonuclear leukocytes were reported to exhibit decreased migration, decreased release of lysozyme and lactate dehydrogenase, and decreased cell viability when exposed to a 0.4 Tesla field for times as short as 30 minutes. This loss in cell viability may explain the loss of functional parameters observed.

Cellular function has been investigated in intense static magnetic fields. Freshly isolated human peripheral blood T-lymphocytes were exposed to static fields up to 6.3 Tesla and evaluated for cell growth and viability. No changes were noted for lymphocytes under standard cell-culture conditions. However, when the T-cells were stimulated with the mitogen PHA, which triggers cell proliferation, cell growth was significantly inhibited compared to controls. Dose response data indicate that this effect was detectable for fields between 4.0–6.3 Tesla, but not for fields below 2.0 Tesla. It is relevant that calcium influx across the cell membrane is required as a second messenger during mitogen activation in the lymphocyte, and a plausible interaction mechanism for this effect on the PHA-treated cells is the inhibition of calcium influx across the cell membrane. These findings are also consistent with the reports dealing with calcium transport of mitogen-activated lymphocytes and calcium-mediated cell function discussed above. A normal human fibroblast cell, DMD-D, and a human malignant melanoma cell line, PS-1273, were also assessed for cell growth and viability in intense static magnetic fields. A 4.7 Tesla magnetic field did not influence cell growth or cell viability of these two cell lines over a 72 hour growth period. However, the authors reported that a significant detachment of the melanoma cells was observed by 12 hours and by 72 hours only a few percent of the cells remained attached; cells could reattach when returned to a nonmagnetic environment. A dose-dependence was observed with fields as low as 0.5
Tesla resulting in 75% detachment at 72 hours. Since cell viability and cell growth were not affected, this field interaction specifically involves cell-surface coupling which mediates cellular adhesion. Rat fibroblasts and osteoblasts were assessed for thymidine and proline incorporation in the presence of a 0.61 Tesla static magnetic field.\textsuperscript{154} Fibroblasts, but not osteoblasts, showed a statistically significant increase in both thymidine and proline incorporation of approximately 0.5–1.0 fold throughout the 10-day exposure protocol; increases in these indices reflect increases in total DNA synthesis and protein synthesis, respectively. In contrast, when explanted neonatal rat calvaria tissue, which was the source of the fibroblasts and the osteoblasts, was used intact in similar tests an inhibition of these indices was detected. The above studies indicate that different cellular model systems can be influenced and can exhibit different sensitivities to static magnetic fields.

Magnetic fields of the order of $10^{-2}$ to $10^{-2}$ T can affect chemical reactions by influencing the electronic spin states of reaction intermediates.\textsuperscript{155–161} Such effects have the potential to lead to biological consequences.\textsuperscript{156–158,162–165} however, it should be cautioned that a magnetic field effect on chemical reaction intermediates in biological systems has not yet been demonstrated under actual physiological condition.\textsuperscript{3}

A relatively simple chemical illustration of the effect involves homolytic cleavage of a chemical bond to produce two radicals.\textsuperscript{9} Since the electrons in the chemical bond are spin-paired in an $S = 0$ or singlet state,\textsuperscript{166} these electrons on the nascent radicals will also have overall singlet character as the radicals separate. Separation is a diffusion-controlled process and there is a high probability that the two radicals will re-encounter each other. If the electrons retain their overall singlet character, a re-encounter is likely to produce recombination. If the electrons have overall triplet ($S = 1$) character, the bond will not reform and the radicals will eventually separate and perhaps participate in other chemical reactions. The transition from singlet to triplet can result from the interaction of the odd electrons of the radicals with the nuclear magnetic moment(s) of the atom(s) on which they have high probability density. This interaction—the magnetic hyperfine interaction—is equivalent to a local magnetic field produced by the nuclei at the location of the electrons. Different local magnetic fields cause the electrons on the radicals to precess at different rates, which destroys singlet phasing and results in triplet formation. However, an applied magnetic field will decouple the electrons and the nuclei, suppressing formation of the triplet state. This enhances the recombination rate and suppresses the other chemical reactions. The decoupling of the electrons and the nuclei will occur when the intensity of the applied field exceeds the effective magnetic fields produced by the hyperfine interactions. Then the electrons will precess in phase about the applied field rather than at different rates about the local fields. This condition is typically satisfied for fields of the order of $10^{-3}$ to $10^{-2}$ T.

A variation of this scheme is proposed to account for the effects of magnetic fields on electron transport in photosynthetic purple bacterial membranes.\textsuperscript{162,164,165} The effects are observed when elements of the transport chain are electrochemically reduced, which is nonphysiological, forcing back transfer of the photoexcited electron. The electron transport sequence can be summarized as follows:

1. $^3\text{A} + \text{photon} \rightarrow ^3\text{A}^\ast$
2. $^3\text{A}^\ast + (\text{B}) \rightarrow ^3\text{A}^\ast + \text{B}^\ast$
3. $^3\text{A}^\ast + \text{B}^- \rightarrow ^3\text{A}^\ast + \text{B}^-$
4. $^3\text{A}^\ast + \text{B}^- \rightarrow ^3\text{A} + (\text{B})$
5. $^1\text{A} + (\text{B}) \rightarrow ^1\text{A} + (\text{B})$

* Homolytic cleavage is the breaking of a covalent, single bond so that one electron from the bond is left on each fragment, resulting in two free radicals with single, unpaired electrons.
(A) corresponds to bacteriochlorophyll dimer and (B) corresponds to bacteriopheophytin. S and T stand for singlet and triplet, respectively, and * stands for the photoexcited state. (1) Bacteriochlorophyll absorbs a photon resulting in electron excitation. (2) Electron transfer to bacteriopheophytin occurs, resulting in positive and negative charges on donor and acceptor, respectively. (3) The positive ion-negative ion pair are initially in a singlet state which can evolve into a triplet state via the hyperfine interaction mechanism. In the blocked transport chain, the ion pair decays by back transfer of the electron to a less energetic state of bacteriochlorophyll. (4) If the ion pair is in the singlet state, back transfer populates the singlet ground state of bacteriochlorophyll. (5) If the ion pair is in the triplet state, back transfer populates an intermediate energy triplet state of bacteriochlorophyll which is detected by a delayed fluorescence method. It was found that the amount of bacteriochlorophyll triplet produced following flash excitation is magnetic field-dependent. Because the photosynthetic apparatus is highly structured and membrane-bound, exchange interactions between the ions also play a role in the formation of the triplet state in (3). Although the experimental conditions cited above are certainly nonphysiological, mechanisms of this kind could conceivably play a role in electron transport in viable biological systems. However, the fact that free radical production in cells, for example, is extremely tightly regulated by multiple redundant systems argues against magnetic field effects on reaction intermediates leading to any adverse, long-lasting consequences.

The action of an intense static magnetic field on the movement of blood or the diffusion of ions, plasma proteins, and paramagnetic molecules has been studied. These investigations address the question of a magnetohydrodynamic effect on flow processes (Figure 3).

As noted in section II, the flow of a conductive element in the presence of a static magnetic field will lead to induced electrical potentials, and the magnetohydrodynamic effect has been predicted on theoretical grounds to produce a reduction in the flow velocity and a compensatory elevation in blood pressure to retain a constant volume flow rate. The occurrence of changes in dynamic pressure could, in principle, impose cardiovascular stress. This might pose a potential problem particularly in biologically-compromised patients during NMR imaging procedures. Monkeys (Macaca cynomolgous) were exposed to a 1.5 Tesla static magnetic field and no measurable alterations in blood pressure were reported. However, an instantaneous, field strength-dependent increase in the EEG signal amplitude at the T wave locus was observed in fields > 0.1 Tesla; this was reversible. This is consistent with

FIGURE 3 Illustration of the magnetohydrodynamic effect, i.e., the drag force generated in a conducting fluid moving in a static magnetic field.
the presence of a magnetically-induced aortic blood flow potential superimposed on a normal T-wave signal. In studies employing humans exposed to static magnetic fields up to 1.0 Tesla for 10-minute sessions, there was no evidence of alterations in local blood flow at the skin of the thumb or at the forearm that were attributable to the applied magnetic fields.\textsuperscript{169} Theoretical studies have estimated the magnetohydrodynamic effects that would be observed at very high intensity fields. A 10 Tesla magnetic field is predicted to change the vascular pressure in a model of the human vasculature by less than 0.2%, and experimental results for the retardation of 15% NaCl flowing transverse to a 2.3 or a 4.7 Tesla field are in general agreement with these predictions.\textsuperscript{170} In addition, the issue of magnetic fields affecting the diffusion of ions and proteins in solution has been addressed theoretically.\textsuperscript{171} A Lorentz force interaction is predicted for charged ions such as Na\textsuperscript{+}, K\textsuperscript{+}, Ca\textsuperscript{2+}, Cl\textsuperscript{-}, and for plasma proteins with a threshold at more than 10\textsuperscript{6} Tesla. In gradient magnetic fields, Maxwell stress interactions are predicted for gradients more than 100,000 T/m for paramagnetic molecules such as FeCl\textsubscript{3} and O\textsubscript{2}, also for plasma proteins. Typical gradient fields in MRI devices are usually less than 100 T/m. However, the movement of deoxygenated erythrocytes and FeCl\textsubscript{3} colloids (greater than 1000 molecules) are easily influenced by normal gradient fields due to a volume effect.

### IV. MUTAGENIC, MITOGENIC, MORPHOLOGICAL, AND DEVELOPMENTAL EFFECTS OF MAGNETIC FIELDS

A large number of papers have been published on this topic and a number of bibliographies, reviews, and symposia have appeared.\textsuperscript{1-3, 172-189} Moreover, a number of interaction mechanisms have been proposed.\textsuperscript{190-196} However, this area is still an empirical science with little elucidation of effects in terms of mechanism. Only some of the more recent reports will be cited here.

Mutagenic effects of chronic exposure to DC magnetic fields have been investigated. A recent review on the genotoxic potential of static magnetic fields assessed a wide range of recent studies. In these studies, the authors conclude that the preponderance of evidence suggests that static magnetic fields do not have a clearly demonstrated potential to cause genotoxic effects.\textsuperscript{197} Mahium et al.\textsuperscript{198} exposed male mice to a magnetic field of up to 1T for 28 days. The mice were subsequently mated to two females per week for up to 8 weeks and the resulting embryos were assayed for viability 10 days later. No significant differences were reported between exposed and sham-exposed (control) groups. Kall and Baum\textsuperscript{199} exposed fruitfly (Drosophila melanogaster) male eggs, larvae, pupae, and adults to fields up to 3.7 T for up to 7 days. After mating with females, broods were tested for induction of genetic damage by the sex-linked, recessive lethal test. No evidence for induction of mutations under the conditions of exposure were reported. Skopek et al.\textsuperscript{200} exposed Salmonella and cultured human lymphoblasts to 10 T magnetic fields for 4 hr. Cells were surveyed for toxic and mutagenic effects with a forward mutation assay. No effects of magnetic field exposure were found for either cell type when compared with sham-exposed controls.

Morphological and developmental effects have been investigated. S. Ueno and colleagues studied the embryonic development of the frog (Xenopus Laevis) by exposing fertilized eggs in Ringers solution to static magnetic fields up to 6.5 Tesla for varying lengths of time.\textsuperscript{201} The time course of early, very rapid cleavage was assessed to determine if static fields affected cell multiplication and differentiation during a 7 hour exposure. No changes were detected in cleavage rates or in the percentage of embryos reaching stage 21 (late neural stage, 22 hour) between control and exposed embryos. In addition, no appreciable defects were observed between control and exposed embryos developing into tadpoles (stage 42) at day 4. In this organism the processes of DNA replication and cell division during early gastrulation, followed by tissue differentiation and organogenesis appears to be insensitive
to intense static magnetic fields. Earlier studies have also reported negative findings on early stages of development of the frog (*Rana pipiens*) exposed to a 0.7 Tesla static magnetic field in a NMR system. Drosophila larvae, in contrast, have been reported to show abnormalities in embryogenesis during exposures to static magnetic fields as low as 1 mTesla. In these studies M. W. Ho and colleagues confirmed earlier reports that exposure of Drosophila to mTesla fields decreases hatching rates, but they extended these observations to specific morphological abnormalities. They reported that exposures for 30 minutes were as effective as 24 hours, and that a flux-density dependence in abnormalities was reported over the range 0-3 mTeslas with no further change in abnormalities between 3-9 mTeslas. The authors state that such weak magnetic fields probably do not influence transembryonic ionic currents in the Drosophila, or do not exert orientation effects on cell membrane components, but that they may be acting via a high degree of cooperativity among molecules involved in the processes of pattern recognition. These recent studies, as well as previous studies, emphasize the important fact that different animal species can display sensitivity to static magnetic fields.

Sikov et al. reported no effects on the development of mice after intrauterine exposure to 1 T during gestation. An earlier study by Nahas et al. had indicated that exposure of rodents to 0.92-0.12 T fields for 1 month resulted in no toxic or histopathological effects. Brewer studied guppies (*Lebistes reticulatus*) chronically exposed to a 0.05 T magnetic field and reported reduction in spawn rate and gestation period in successive generations exposed to the field. However, field effects were not permanent; reproduction eventually returned to normal when fish were removed from the magnetic field. Mild et al. studied development of frog (*Xenopus laevis*) embryos exposed at 0.25 T for periods up to 1 week, at temperatures just above the threshold for development in the embryos. If the effect of the magnetic field is equivalent to a reduction in temperature, exposed embryos should not have developed. However, no differences between development of exposed embryos and unexposed controls were reported. Previous studies had indicated effects of magnetic field exposure on development of frog embryos. Strand et al. reported enhancement of fertilization following exposure of trout sperm, ova, or both to 1 T magnetic fields.

Frazier et al. investigated mammalian cell cultures continuously exposed to magnetic fields of 0.1 or 0.3 T through 80 cell doublings. No mitogenic effects of the field were reported when doubling times of exposed cells were compared to controls. Differences in plating efficiencies between exposed cells and controls were cited and ascribed to an as-yet unexplained increased clumping of exposed cells. Exposure of frozen cells at 1 T did not result in changes in cell morphology as reported earlier. Cultured cells from human bronchogenic carcinoma and from Burkitt Lymphoma were exposed to DC magnetic fields up to 1.15 T by Chandra and Stefani. They reported that growth characteristics were unaffected by exposure. *In vivo* exposure of mouse tumors did not cause retardation of growth of the tumor. Leitmannova et al. reported changes in morphology of aged red blood cells in magnetic fields.

Moore studied growth of five species of bacteria and a yeast in DC and slowly varying magnetic fields up to 0.09 T. He reported stimulation or retardation of growth depending on the field strength, frequency, and organism. A number of previous studies had indicated that growth of bacteria and yeasts could be altered by static magnetic fields.

Electrophysiological effects of static magnetic fields have been investigated. Blatt and Kuo reported no change in the action potential in the interpodal cells of the fresh water alga *Nitella* exposed to fields up to 2 T. These cells have bioelectrical activity and previous studies had indicated a reduction of the action potential in magnetic fields. Extended exposure at 1.6 T was not toxic for cells. Edelman et al. reported an increase with time in the amplitude of the compound action potential of stimulated frog sciatic nerve when fields up to 0.71 T were applied perpendicular to the nerve. Fields applied parallel to the nerve produced no changes. However, Gaffney and Tenforde reported no changes in electrical
conduction in frog sciatic nerve in fields up to 2 T and suggested that results of Edelman et al.\textsuperscript{223} reported electrical changes in cells in the pineal glands of guinea pigs when exposed to magnetic fields of the order of $10^{-4}$ T. Raybourn\textsuperscript{224} reported that $10^{-3}$ to $10^{-2}$ T fields acutely reduce electroretinographic responses in turtle retina, but do not reduce retinal sensitivity. This effect might involve magnetic field effects on chemical reactions (see Section III).

Studies by Rosen indicate that a significantly weaker magnetic field of 0.12 T can decrease both the amplitude and the variability of a visually evoked response in the adult cat striate cortex.\textsuperscript{226} This effect was observed to have a latency period since the effect developed slowly 50 seconds after the field was turned on and persisted for minutes after the field was turned off. This group also reported that 0.12 T magnetic fields altered the spontaneous discharge frequency and discharge patterns of principal cells in the cat’s lateral geniculate body.\textsuperscript{227} Decreases in frequency and in short interspike intervals were detected, with a gradual onset of the effects. The authors hypothesized in both reports that calcium ions may be involved in this response since latency is consistent with an effect on neurotransmitter release which is calcium dependent. In contrast to these results, when the somatosensory evoked potentials of the cat were studied in fields up to 4.7 T no statistically significant changes were observed in EEG intensity, or EEG frequency index that correlated with field magnitude although substantial variations in these parameters were observed during experiments.\textsuperscript{228} The authors interpreted this as reflecting spontaneous fluctuations of vigilance and not as effects of the static magnetic field.

Studies on miniature end plate potentials (MEPPs) in 0.12 T static magnetic fields have been reported.\textsuperscript{229} Marine neuromuscular junction preparations maintained in a 0.12 T magnetic field exhibited a modest increase in frequency at temperatures below 34°C and a prominent decrease in frequency at temperature above 35°C. When calcium was removed from the media this temperature-dependent field response was not observed. The author interpreted the data to indicate that a phase transition was operating and that the applied magnetic field was sufficient to orient diamagnetic elements of the presynaptic membrane and alter calcium transfer. Further studies with this model system indicate that a minimum of 50 seconds and a maximum of 150 seconds of field exposure is associated with MEPP inhibition at 35°C.\textsuperscript{229} This interpretation is consistent with a slow orientation of diamagnetic domains that might be coupled to neurotransmitter release in this system.

Static magnetic fields affect electrocardiograms in mammals.\textsuperscript{167,168} Alterations in the T wave of rats, rabbits, and baboons are reported at exposures above 0.3 T, and are due to the potentials associated with the flow of blood in the magnetic field, as discussed above. There are apparently no chronic physiological effects associated with this phenomenon.

Ripamonti et al.\textsuperscript{230} studied the effect of magnetic field exposures up to 12.5 T on the responses of the contractile protozoan \textit{Spirostomum ambiguum} to the toxic substance 2,2’ dipiridyldisulfide. Magnetic field exposure reportedly diminished the ability of the organism to survive the drug and lengthened the extension phase of the contraction cycle. It was hypothesized that interactions of the magnetic field with cellular membranes alters the regulation of calcium transients. There were no toxic effects of exposure to magnetic fields without the drug. Bucking et al.\textsuperscript{231} had previously reported that magnetic field exposure affected the force of contraction of isolated muscle, which also involves regulation of intracellular calcium.

deLorge\textsuperscript{232} reported that low-intensity magnetic fields have no measurable effects on operant behavior in monkeys. However, experiments at high magnetic fields showed a suppression of a learned response above a threshold between 4.6 and 7.0 T. Davis et al.\textsuperscript{234} reported no behavioral alterations in mice exposed to 1.5 T magnetic fields. Further data on the effects of very intense magnetic fields come from NMR studies on perfused, whole
organisms. Fossel et al. noted that exposure of perfused rat hearts at 6.4 T did not alter either pressure development or heart rate.

A number of studies report effects in animals exposed to static magnetic fields. When mice were chemoinmobilized and exposed to a 1.4 Tesla static magnetic field the activity of thymidine kinase in bone marrow cells was influenced. The effect depended on animal body temperature with an increase in activity at 27–29°C, and a decrease in activity at 37–39.5°C. A dose-response was observed from 0.2–1.4 Tesla for the low temperature effect. A thirty-minute exposure was required for full expression of the effect at 1.4 Tesla, which was reversible within 5–10 minutes. Of interest is the observation that these effects were not observed in moving rats, in cell suspensions, or in enzymes in solution. The authors interpreted these findings to indicate that a complex, intact structure such as the cell membrane is likely involved in this interaction in contrast to a magnetic field effect on individual free enzymes or other molecules in solution such as free radicals. Another study employed immobilized rats, but a ventilated restraining chamber was used instead of a chemorestraint during exposure to a 1.0 Tesla static magnetic field. Rats were exposed to the magnetic field for 30 minutes on each of ten consecutive days. No significant differences were reported in blood alkaline phosphatase, acid phosphatase, calcium ion concentration, and phosphate ion concentration between control, sham-exposed, and magnetic field-exposed animals. The authors suggested that these results support the idea that short-term exposures to 1.0 Tesla fields do not alter physiological mechanism of bone mineralization.

The effects of static magnetic fields on in vivo immunity has been studied in rats implanted with micromagnets (600 Gauss, 60 mTesla) in the skull in the frontal, parietal and occipital regions. After 20 days, rats were either challenged with antigen or not challenged: both implanted-micromagnet and control animal groups were subsequently assessed for immune status for 34 days. The implanted micromagnets were reported to immunopotentiate both humoral and cell-mediated immune responses, with highest performance obtained from occipital exposure for a total period of 24 days. The use of implanted magnets in bioeffects studies is a recent development; there is a limited database for comparison of results. Several such studies have been reported in the area of permanent magnet devices for retaining dental prosthetic appliances, discussed above. The use of implanted magnets raise challenging questions regarding biocompatibility and in vivo dosimetry to target tissues.

A survey of workers exposed to intense magnetic fields was conducted by Beischer who found no adverse effects of short exposures to fields up to 0.5 T. Epidemiological studies of magnetic field effects in humans are being conducted presently by Badinger et al. and by the National Radiological Protection Board in the U.K. Reviews concerned especially with potential hazards of magnetic field exposure associated with NMR imaging have been published, as discussed above.

Finally, nature has conducted an experiment over geologic times on life in a substantial magnetic field. Magnetotactic bacteria are sediment-dwelling bacteria that contain particles of Fe₃O₄ that cause them to be oriented in the geomagnetic field (see Section V). These particles produce strong intracytoplasmic magnetic fields and field gradients in the bacterium. The fields can be as large as several tenths of a Tesla near the surface of the particles. Thus, these bacteria carry out all of their cellular and metabolic functions in intracellularly generated magnetic fields, and have presumably done so for billions of years.

V. THE GEOMAGNETIC FIELD IN THE ORIENTATION AND HOMING OF ORGANISMS

At the end of the last century, Kreid published a report describing magnetic field effects on orientation in crabs. The experimental design was contrived to produce an effect and so does not test behavior in the natural environment of crabs, but could provide a paradigm for
effects in other organisms. Crabs periodically molt and form a new exoskeleton. In the process of molting, they also lose their statoliths, the dense particles that form part of the vestibular system. They subsequently pick up particles of sand to serve as new statoliths. Kreidl took newly molted crabs and placed them in an aquarium in which only magnetic particles were available. The crabs indeed placed magnetic particles in their ear labyrinths. When the crabs were subsequently approached with a bar magnet, they adopted an orientation that could be correlated with the resultant of the magnetic and gravitational forces on the particles. Electrophysiological responses to magnetic field stimulation have been recorded in crayfish with ferrite statoliths.242

Since Kreidl's experiment, magnetic field effects on orientation and homing have been reported for a very diverse group of normal organisms, including bacteria,240 algae,243 snails,244 planaria,245 honeybees,246 fish,247 mollusks,248 amphibians,249 homing pigeons,250 migratory birds,5,251 mammals,252 and humans.253-255 In addition, conditioning experiments on pigeons,256 skates,257 tuna258 and honeybees259 have demonstrated the ability of these organisms to sense magnetic fields. Cetacean strandings have been correlated with geomagnetic field anomalies.260 They have been reported in several organisms.262,263

These observations imply existence of an organ or organelle that transduces magnetic field intensity, direction or gradient information. Three interaction mechanisms have been proposed:

1. detection by the organism of the electric field induced by Faraday effect as the organism moves through the magnetic field;
2. interaction of the magnetic field with magnetic material in the organism;
3. effect of the magnetic field on chemical reactions or absorption of photons.

The first mechanism is apparently operative in marine sharks, skates, and rays.255 which are sensitive to electric fields as low as $5 \times 10^{-7}$ V/m in sea water.264 They detect the electric fields through the **ampullae of Lorenzini**, which are long, conductive channels that connect electrically sensitive cells in the snout with pores on the skin. Flowing ocean currents or motion of the animal through the geomagnetic field induce voltage gradients with sign and magnitude depending on orientation, which are, in general, above the sensitivity threshold of the animal. Kalmijn255 demonstrated that skates could be trained to use magnetic fields of the order of the geomagnetic field as an orientational cue. Brown et al.266 used electrophysiological measurements to show that the **ampullae of Lorenzini** can detect variations in the geomagnetic field. Jungeman and Rosenblum267 have considered the possibility of the magnetic induction mechanism for an animal in air. They concluded that a circular, electrically conducting loop millimeters in diameter, would be required to overcome thermal noise, with voltages induced by changes in magnetic flux in the loop as the animal changes its heading.

Evidence for orientation by the second mechanism was obtained for homing pigeons in the classical experiment of Keeton.268 Keeton glued small bar magnets to the backs of the heads of a group of homing pigeons and compared their homing ability with that of a group of control birds carrying brass weights. Under sunny skies, both groups oriented and homed equally well when released from unfamiliar sites many miles from the home loft, but, under overcast skies, when the birds could not see the sun, the orientation of the birds carrying magnets was disrupted, whereas control birds oriented normally. Subsequently, Walcott and Green269 used Helmholtz coils attached to the heads of pigeons to change the orientation of the birds under overcast conditions. The orientation depended on the direction of the magnetic field, as determined by the direction of current in the coils. Further studies suggest that the
magnetic compass sense in pigeons and migratory birds is sensitive to the inclination of the geomagnetic field.

Pigeon orientation is also affected by magnetic anomalies and magnetic storms. The experimental results suggest that in addition to a magnetic compass, a homing pigeon may have a "map" or position finding system that includes magnetic cues. The results have been reviewed by Walcott, Gould, Able, Griffin, and Wiltschko. Although attempts to observe magnetic sensitivity in pigeons by cardiac response have not been successful, Bookman reported training pigeons to detect the presence of magnetic fields in a flight cage.

Walcott et al. dissected pigeons with nonmagnetic tools and found magnetic material in head and neck sections. Most of the magnetic material was localized in a piece of tissue between the dura and the skull. Each pigeon had an inductive, remanent moment of $10^{-8}$ to $10^{-9}$ A·m$^2$, which disappeared at $575^\circ$C, indicating Fe$_3$O$_4$. Presti and Pettigrew found magnetic material in the neck musculature of pigeons and migratory, white-crowned sparrows but did not find localized magnetic materials in the heads. Thus, the connection between the magnetic material and magnetic sensitivity was not definitely established. Yorke, Kirschvink and Gould, and Presti and Pettigrew have speculated on the possible role of magnetite, Fe$_3$O$_4$ in a magnetic sensor. Yorke pointed out that if a pigeon could somehow measure the total magnetization of its ensemble of magnetic particles, there is enough magnetic material present to indicate the field direction with high accuracy.

Migratory birds are also able to use the geomagnetic field as a compass to find and maintain direction (see reviews by Able and by Wiltschko). As in pigeons, the compass is an inclination compass referring to dip angle, rather than polarity. Beason studied the bobolink (Dolichonyx oryzivorus), a nocturnal migrant that integrates information from several sources to determine the preferred direction. Magnetic and histological studies revealed the presence of magnetic material, probably magnetite, in the ethmoidal region. Electrophysiological studies have revealed that the trigeminal nerve system of the bobolink responds to changes in magnetic field intensity and direction. Responses to magnetic field changes were also recorded in the optic tectum and pineal gland. It has been suggested that there are two separate magnetic systems, with at least one involving magnetite for transduction of magnetic field information.

A possible connection between magnetic material and magnetic field effects on behavior was also found in honeybees. The behavioral effects have been reviewed by von Frisch. Martin and Lindauer and Gould. Honeybee workers communicate the location of a food source to other workers in a hive by means of a "waggle dance" on a vertical honeycomb. The angle between the direction of the dance and the vertical direction indicates the angle between the food source and the sun. Consistent errors in the dance angle occur which vanish when the magnetic field in the hive is nullled by means of external coils. In anomalous situations where bees are made to dance on horizontal surfaces, after an initial period of disorientation they dance along the eight magnetic, compass directions (N, NE, E, SE, etc.). If the geomagnetic field is nullled, the dances become disoriented. Evidence has also been reported that bees can use the diurnal variations in the geomagnetic field to set their circadian rhythms.

Walker and Bitterman have reported conditioning experiments that demonstrate the sensitivity of free-flying bees but not stationary bees to local magnetic anomalies. The threshold sensitivity was determined to be ca. 0.6% of the background geomagnetic field. Magnetized wires attached to the bees abdomen disrupted the ability of the bees to detect the anomalies.

Gould et al. have found that honeybees contain magnetic material. They measured an average, induced remanent moment of about $2 \times 10^{-9}$ A·m$^2$ per bee, distributed between single-magnetic-domain and superparamagnetic particles. The magnetic material was mostly localized in the abdomen. Kuterbach et al. found bands of cells around the abdominal
segments in honeybees that contained numerous iron-rich granules. The granules were primarily a hydrous iron oxide, which can be a precursor in the precipitation of Fe₂O₃.²⁸⁹

Evidence for magnetic sensitivity has been obtained for bony fish including several species of salmon and trout. Walker observed unconditioned and conditioned responses to local magnetic field anomalies in actively swimming yellowfin tuna (Thunnus albacares).²⁵⁸ Magnetic studies of tuna indicated the presence of magnetite particles associated with the ethmoid tissue in the head.²⁹³ Magnetite particles extracted from the ethmoid region in sockeye salmon were found to be single magnetic domains arranged in strings.²⁹² Single magnetic domain particles have also been found in chinook salmon.²⁹³

In addition to the cases cited above, magnetic inclusions, principally Fe₂O₄, occur widely in the biological world.²⁹⁴-²⁹⁵ Magnetic material has been reported in organisms as diverse as dolphins,²⁹⁶ butterflies,²⁹⁷ tuna,²⁹⁸ green turtles,²⁹⁹ marine crustacea,³⁰⁰ bacteria,³⁰¹ and humans.³⁰² The first identification of Fe₂O₄ in an organism was by Lowenstam,³⁰³ who found it in the tooth denticles on the radulae of chitons, a group of mollusks. Fe₂O₄ is very hard as well as magnetic which is advantageous to chitons since they scrape algae off rocks. Kirschvink et al.³⁰⁸ have recently reported the detection and identification of magnetite particles in human brain tissue. The concentration is ca. 5 nanograms per gram of tissue, out of a total iron concentration of ca. 200 micrograms per gram tissue. The neurophysiological function or significance is not known, but a considerable fraction is in the form of single-magnetic domain cubo-octahedra.

The best documented connection between magnetically sensitive behavior and the presence of magnetic material is for motile, aquatic bacteria that orient and migrate along geomagnetic field lines.¹²²,²⁴⁰,³⁰¹,³⁰² This behavior, magnetotaxis, is exhibited by a number of freshwater and marine bacteria; the diversity of morphological types suggests that the phenomenon is a feature of a number of bacterial species.³⁰¹,³⁰³ Magnetotactic bacteria are common in the sediments of almost any aquatic environment. They are also localized in horizontal plates at specific depths in water columns with vertical chemical and redox gradients, principally at the anoxic-anoxic transition zone.³⁰⁴ Several magnetotactic bacteria have been cultured axenically.³⁰⁵,³⁰⁶

Individual magnetotactic bacterial cells contain intracytoplasmic mineral particles of magnetic (Fe₂O₄),²⁹⁸,³⁰⁶-³⁰⁹ or greigite (Fe₂S₄),³⁰⁸-³¹⁰ usually arranged in chains. Greigite is isostructural with magnetite and is also magnetically ordered at ambient temperatures. The particles in a given species or strain are characterized by a narrow size distribution and a specific crystalline habit.³¹⁰ A number of particle habits for both magnetite and greigite have been elucidated by high resolution transmission electron microscopy and electron diffraction studies.³¹⁰,³¹¹ Some of the habits are equilibrium forms, i.e., they preserve the symmetry of the fcc unit cell, while others are of lower symmetry, implying the relative acceleration or retardation of the growth of certain crystal faces. While most magnetotactic bacteria contain only one particle type, one organism, a multicellular prokaryote,³¹² contains particles of nonmagnetic pyrite, FeS₂, as well as greigite.³⁰⁸ Another organism, a large marine rod, contains particles of magnetite and greigite with different morphologies, arranged within the same chain of particles.³¹³

The mineral particles in magnetotactic bacteria are enclosed in a membrane within the cell;³¹⁴ a particle and its enveloping membrane is known as a magnetosome.³¹⁵ In Aquaspirillum magnetotacticum, the membrane has a protein profile that is different than that of the cytoplasmic membrane. The membranes form or are attached to an ultrastructural entity that organizes the magnetosomes into chains, and holds the particles in a fixed position within the cell.

While the details of magnetosome chain formation in magnetotactic bacteria are unknown, it is thought to involve two separately controlled processes, an ultrastructural process associated with chain assembly, and a mineralization process associated with particle formation.³¹³
Phylogenetic analyses of magnetite- and greigite-forming bacteria show that the two groups are distantly related, suggesting that different mineralization processes are involved in the two mineral particle types. \[316\]

The magnetosome chain is a hierarchical structure that constitutes a permanent magnetic dipole within each cell. \[317\] The magnetosome particles are typically with the single-magnetic-domain size ranges for magnetite and greigite. When arranged in chains, the individual particle moments are oriented parallel to each other along the chain axis. The orientation of the net cellular dipole moment by the geomagnetic or ambient magnetic field causes the cell to migrate along the field lines. Thus magnetotaxis is a passive process and each cell is, in effect, a mobile biomagnetic compass needle. Cells can use other sensory responses, such as aerotaxis in conjunction with magnetotaxis efficiently to find and maintain position in chemical gradients. \[318\]

The best documented behavioral evidence for the involvement of light in magnetic orientation is for Eastern red-spotted newts (\textit{N. viridescens}). The ability of these organisms to use the geomagnetic field for shoreward orientation was studied by Phillips, \[319,320\] who investigated whether the magnetoreception mechanism of shoreward-orienting newts is axial or polar, i.e., whether it determines the magnetic axis, or determines a preferred direction along the magnetic axis. This was done by studying the orientation responses of the animals following inversion of the vertical component of the ambient magnetic field. It was found that newts that had been trained to associate shoreward orientation with the ambient magnetic field changed their orientation by about 180 degrees in response to inversion of the vertical component of the field, compared to controls for which the vertical component was not inverted. Since inversion of the vertical component left the horizontal component of the ambient field unchanged, if the magnetoreception mechanism were polar, the orientation of the animals should not have changed. The behavioral response of the newts therefore suggests that the magnetoreceptor mechanism in shoreward orientation is axial, and that the animal relies on the magnetic field inclination to discriminate between the two directions along the horizontal projection of the magnetic axis.

Newts also use geomagnetic field cues in homing behavior when displaced long distances. In this case, the mechanism appears to be polar, because inversion of the vertical component of the ambient field did not affect homing orientation. Thus newts apparently have two magnetoreception modalities.

Phillips subsequently studied red-spotted newts which were trained in natural (full spectrum) light to maintain a consistent orientation with respect to the ambient magnetic field, by association with orientation toward an artificial shore. They were subsequently tested in an arena where the horizontal component of the ambient magnetic field, as well as the wavelength composition of the ambient light, could be varied. Under full spectrum illumination, newts maintained the same orientation to the horizontal component of the ambient magnetic field as in the training regime. In the dark, the newts were randomly oriented. Under monochromatic short wavelength light (400 or 450 nm), the newts were oriented as under full spectrum light. However, under long wavelength light (500, 550 or 600 nm), the newts were oriented at 90 degrees (anti-clockwise) to the full spectrum direction. Under 475 nm light, newts were oriented at random. When newts were trained under long wavelength (>500 nm) light, they maintained the same orientation with respect to the ambient magnetic field when tested under long wavelength light, but were oriented at 90 degrees (clockwise) to the long-wavelength direction when tested under full spectrum conditions. The experimental results can be understood in terms of magnetic field modulation of photoreceptors by light. Phillips has hypothesized two types of light dependent receptors, or two spectral mechanisms in the same cell, with long- and short-wavelength response respectively, that are sensitive to the ambient magnetic field. The two receptor types, or mechanisms, give antagonistic
neural inputs, but the short-wavelength receptor, or mechanism, has greater light-intensity sensitivity and predominates under full spectrum conditions.

Evidence for wavelength-dependent effects of light on magnetic compass orientation in fruit-flies (Drosophila melonogaster) and in migratory birds has also been reported. On the other hand, Lohmann has reported that loggerhead and leatherback sea turtle hatchlings can orient to the geomagnetic field in complete darkness. Magnetic orientation in darkness also occurs in other organisms. In turtles, magnetic orientation is axial as in newts. Lohmann has proposed that magnetoreception in darkness could still involve photoreceptors based on magnetic field dependent biochemical reactions. However, there is no current evidence for magnetic field modulation of light independent biochemical reactions in the retina or elsewhere.

Lohmann and Willows studied the spontaneous orientation of a marine mollusk, the nudibranch Tritonia diomedea, under dark conditions. The animals spontaneously adopted a significant, preferential orientation with respect to the geomagnetic field. The orientation direction varied with the lunar phase. When the horizontal component of the geomagnetic field was cancelled with a coil system, the animals' preferential orientation vanished. Further experiments with migration in a maze showed that T. diomedea can use the geomagnetic field for directional cues. The organism has large, individually identifiable neurons, and intracellular electrical recordings from at least one neuron have shown electrophysiological responses to changes in the ambient (earth-strength) magnetic field, such as rotation of the field. It has not yet been determined whether this neuron is the primary magnetoreceptor or part of the signal pathway from another source.

In conclusion, a magnetoreception organ or even mechanism has not been clearly identified in any organism except for magnetotactic bacteria and skates and rays. However, the recent results on the involvement of light in magnetoreception, and on electrophysiological responses to changes in the ambient magnetic field are very promising for finding a magnetoreceptor and elucidating a magnetoreception mechanism. It may turn out that there is more than one magnetoreception modality, based on magnetic particles, magnetic effects on chemical reactions, light absorption or some combination of the three.

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APPENDIX: ELECTROMAGNETIC UNITS AND DEFINITIONS

Purcell gives an excellent discussion of magnetic field concepts; for a review of magnetic measurements see Morrisey and Foner. In discussing the interactions of static magnetic fields with materials most workers use the centimeter-gram-second (cgs)-electromagnetic units (emu). In the SI system, the magnetic flux density is defined by

\[ B = \mu_0 H \]
\[ B = \mu_0 (H + M) \]  
(A1)

in vacuum and in a material medium, respectively. \( H \) is the magnetic field intensity and \( M \) is the magnetization per unit volume. The permeability of free space is given by

\[ \mu_0 = 4\pi \times 10^{-7} \ \text{H/m} \]  
(A2)

where \( H/\text{m} \) (henry per meter) is equivalent to weber per meter per ampere. The volume magnetization of diamagnetic and paramagnetic materials is related to the magnetic field intensity by the magnetic susceptibility \( \chi \)

\[ M = \chi H \]  
(A3)

In the SI system, \( \chi \) is a dimensionless quantity. In magnetically ordered materials, \( M \) is a complex function of \( H \) and can have finite values even at \( H = 0 \).

In the cgs-emu system, \( \mu_0 = 1 \) and

\[ B = H + 4\pi M \]  
(A4)

Some of the relations between emu and SI units are as follows:

- Magnetic flux density \( B \): 
  \[ 1 \ \text{gauss} = 10^{-4} \ \text{Tesla} \]
- Magnetic field intensity \( H \): 
  \[ 1 \ \text{oersted} = (10^3/4\pi) \ \text{amp/m} \]
- Magnetic moment \( \mu \): 
  \[ 1 \ \text{emu} = 1 \ \text{erg/gauss} = 10^{-3} \ \text{amp\cdot m}^2 \]
- Magnetization \( M \): 
  \[ 1 \ \text{emu/cm}^3 = 10^3 \ \text{amp/m} \]
- Magnetic susceptibility \( \chi \): 
  \[ \chi(\text{emu}) = (1/4\pi) \chi(\text{MKS}) \]

In the emu system, Faraday's law of magnetic induction is

\[ \epsilon = -10^{-8} \frac{d\Phi}{dt} \]  
(A5)

where the emf (electromotive force) \( \epsilon \) (volts) is induced in a conducting loop of area \( A \) normal to \( B \), and the magnetic flux

\[ \Phi = BA \]  
(A6)

The emf can be produced by a time-varying field in a stationary loop or by a loop whose area perpendicular to the field direction is changing with respect to a static magnetic field.

The magnetic moment, magnetization, and magnetic susceptibility of materials are expressed on a unit weight, unit volume, unit mole, unit atom, or unit molecule basis. Magnetic moments and magnetization have the units emu, gauss, cgs, or ergs per gauss in the emu system, with 1 emu = 1, G = 1, cgs = 1, erg/G. For example, the saturation magnetic moment per unit volume, or magnetization, of \( \text{Fe}_3\text{O}_4 \) is 480 emu/cm\(^3\), or 92 emu/gram. The conversion factor is the density. Magnetic moments of atoms and molecules are often expressed as bohr magnetons (\( \mu_B \)) with 1 \( \mu_B = 0.927 \times 10^{-21} \) ergs/G. The electron has a magnetic moment of 1 \( \mu_B \). \( \text{Fe}_3\text{O}_4 \) has a magnetic moment of 4 \( \mu_B \) per formula unit.

The free energy of magnetic dipoles or of materials with permanent, macroscopic magnetization \( M \) oriented at angle \( \Theta \) in a magnetic field of flux density \( B \) is

\[ E_m = -M \cdot B = -MB \cos \Theta \]  
(A7)

In a homogeneous magnetic field the free energy is a minimum when \( \cos \Theta = 1 \), i.e., \( M \) is
parallel to $\mathbf{B}$. In an inhomogeneous magnetic field, additional lowering of the free energy comes from translational motion of the material toward increasing field strength. The translational force along the gradient is

$$ F_x = M \frac{d\mathbf{B}}{dx} $$

\hspace{1cm} (A8)

where the magnetic field gradient is taken along the $x$ axis.

For materials without a permanent dipole moment, a magnetic field will induce a magnetization $\mathbf{M}$, where

$$ \mathbf{M} = \chi \mathbf{H} $$

\hspace{1cm} (A9)

$\chi$ is the susceptibility tensor with units emu/G or ergs/G$^2$. For diamagnetic materials, $\chi$ is small and negative and is generally independent of temperature. For example, H$_2$O has an isotropic volume magnetic susceptibility $\chi_v = -0.4 \times 10^{-7}$ emu/(G·cm$^3$). In paramagnetic materials, i.e., materials with unpaired electrons, $\chi$ is larger in magnitude, positive in sign, and is generally temperature-dependent. At ambient temperatures, typical paramagnets have susceptibilities that follow the Curie Law:

$$ \chi = N \frac{\mu_{\text{eff}}^2}{3k_B T} $$

\hspace{1cm} (A10)

where $N$ is the number density of paramagnetic atoms, $\mu_{\text{eff}}$ is the effective magnetic moment per atom and $k_B T$ is Boltzmann's constant times temperature. According to quantum mechanics, the saturation magnetic moment of an atom or molecule is proportional to the total angular momentum

$$ \mu = g \mu_B J $$

\hspace{1cm} (A11)

where $\mu_B$ is the Bohr magneton, $g$ is the proportionality constant known as the g-factor, and $J$ is the angular momentum quantum number. If we consider spin angular momentum only, $g = 2$. The effective magnetic moment is then

$$ \mu_{\text{eff}} = [g^2 S (S + 1) \mu_B^2]^{1/2} $$

\hspace{1cm} (A12)

In a hypothetical example, if every water molecule had an unpaired electron spin ($S = 1/2$), the magnetic moment per molecule would be $1 \mu_B$ and the paramagnetic susceptibility per cubic centimeter at 300 K would be $2.2 \times 10^{-6}$ emu/G. The total susceptibility would be the sum of paramagnetic and diamagnetic contributions, giving $2.16 \times 10^{-6}$ emu/G. In some cases the diamagnetic susceptibility contribution can be larger than the paramagnetic contributions. This is often the case in large biological molecules that have a single or a few paramagnetic atoms.

In a magnetic field the magnetic energy is given by

$$ E_m = -\langle \mu_{\text{eff}} \rangle \cdot \mathbf{H} $$

\hspace{1cm} (A13)

where the magnitude and direction of the induced moment depend on the orientation of the molecule in the field. If the susceptibility is isotropic, the induced moment is always parallel to $\mathbf{H}$ and...
\[ E_m = -(1/2) \chi H^2 \]  

(A14)

If \( \chi \) is isotropic, there are no translational forces in a homogeneous magnetic field. In an inhomogeneous magnetic field, the material will experience a translational force in the direction of increasing or decreasing field strength depending on the sign of \( \chi \)

\[ F_x = \chi V H \frac{dH}{dx} \]  

(A15)

where \( \chi \) is the susceptibility per unit volume and \( V \) is the volume. Diamagnetic materials move in the direction of decreasing field strength while paramagnetic (and ferro or ferrimagnetic) materials move toward increasing field strength. As discussed in Section II, this is the basis of a method for separating diamagnetic from paramagnetic materials. In suspensions or solutions, the force depends on the difference in susceptibility between the material and the medium.

Anisotropic materials require two or, at most, three independent parameters to specify the magnetic susceptibility. In the most general case,

\[ E_m = -(1/2) (\chi_x H_x^2 + \chi_y H_y^2 + \chi_z H_z^2) \]  

(A16)

where \( x, y, \) and \( z \) denote the eigenvectors of the diagonalized susceptibility tensor. These three vectors often correspond to molecular symmetry axes. In addition to translational forces in inhomogeneous fields, there are rotational forces in homogeneous fields. For example, benzene has an inplane susceptibility \( \chi|| = -4.5 \times 10^{-7} \text{ emu/G-cm}^3 \) (\( = -5.7 \times 10^{-5} \text{ in SI units} \)) and a susceptibility normal to the plane \( \chi\perp = -12 \times 10^{-7} \text{ emu/G-cm}^3 \) (\( = -1.5 \times 10^{-5} \text{ in SI units} \)). The molecule will experience a torque, tending to align its plane parallel to the field direction. In general, any molecule or molecular assembly with anisotropic diamagnetism will tend to align so that the least negative susceptibility direction is parallel to the applied field.

In anisotropic paramagnets the highest (positive) susceptibility direction will tend to align parallel to the field. If \( \chi|| \) is the susceptibility along the minimum energy direction and \( \Theta \) is the angle between that direction and the applied field

\[ E_m = - \langle 1/2 \rangle [\chi||H^2 - \Delta \chi H^2 \cos^2 \Theta] \]

\[ \Delta \chi = \chi|| - \chi\perp \]  

(A17)

where by definition \( \Delta \chi > 0 \) and \( E_m \) is minimized when \( \Theta = 0 \). The degree of orientation of an ensemble of molecules at a given field strength and temperature can be calculated from statistical mechanics. The angular distribution function \( F(\Theta) \), which specifies the probability that a molecule has an equilibrium orientation at angle \( \Theta \) with respect to \( H \), can in general be expanded in terms of \( \cos^n \Theta \). For molecules with cylindrical symmetry, odd terms in \( \Theta \) vanish. Then a convenient measure of the degree of orientation is the average value of the second Legendre polynomial

\[ \langle P_2 (\cos \Theta) \rangle = \langle (3/2) \cos^2 \Theta - (1/2) \rangle \]  

(A18)

From statistical mechanics,

\[ \langle P_2 (\cos \Theta) \rangle = \int \left( 3/2 \cos^2 \Theta - 1/2 \right) \exp(-1/2 \Delta \chi H^2 \cos^2 \Theta/k_B T) \, d\cos \Theta / Z \]  

(A19)

where \( Z \) is the partition function. Complete alignment means \( \langle \cos^2 \Theta \rangle = 1 \) and \( \langle P_2 (\cos \Theta) \rangle = 1 \).
1. For random orientation in three dimensions \( \langle \cos^2 \Theta \rangle = 1/3 \), hence \( \langle P_2(\cos \Theta) \rangle = 0 \). Even for molecules such as benzene with large diamagnetic anisotropies, thermal agitation will overcome magnetic alignment and the equilibrium alignment will be small. However, if N molecules are contained in an ordered array or aggregate, \( \Delta \chi \) for the single molecule would be replaced by \( N \Delta \chi \) in the exponential in Equation 19, resulting in substantial enhancement of alignment.

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